

amino acid sequence which extends across the membrane as in the case of cell surface proteins, including many receptors. The transmembrane peptide sequence may be extended to span part or all of an extracellular and/or intracellular domain as well. Alternatively, the membrane retention domain may be a lipid membrane retention domain such as a myristoylation or palmitoylation site which permits association with the lipids of the cell surface membrane. Lipid membrane retention domains will usually be added at the 5' end of the coding sequence for N-terminal binding to the membrane and, proximal to the 3' end for C-terminal binding. Peptide sequences involving post-translational processing to provide for lipid membrane binding are described by Carr, et al., PNAS USA (1988) 79, 6128; Aitken, et al., FEBS Lett. (1982) 150, 314; Henderson, et al., PNAS USA (1983) 80, 319; Schulz, et al., Virology (1984), 123, 2131; Dellman, et al., Nature (1985) 314, 374; and reviewed in Ann. Rev. of Biochem. (1988) 57, 69. An amino acid sequence of interest includes the sequence M-G-S-S-K-S-K-P-K-D-P-S-Q-R (SEQ ID NO 1). Various DNA sequences can be used to encode such sequences in the various fusion proteins of this invention. Other localization domains include organelle-targeting domains and sequences such as K-D-E-L (SEQ ID NO 2) and -H-D-E-L (SEQ ID NO 3) which target proteins bearing them to the endoplasmic reticulum, as well as nuclear localization sequences which are particularly useful for fusion proteins designed for (direct) transcription regulation. Various cellular localization sequences and signals are well known in the art.

• Beginning on page 78, line 28, (ending on page 80, line 6) replace the section in the specification with the following:

Oligos for construction of calcineurin A fragments:

CNA sequence is in bold

hCNA 5' PCR Oligo Start at residue 12

Junk Xho1
5' cggg ccc ccc ctc gag **tct acg acc gac agg gtg gtg aaa gc** 3' (SEQ ID NO 4)

Note: g->t is a silent mutation that destroys the SalI site.

hCNA 5' PCR Oligo Start at residue 340

Junk Xho1
5' atat aaa tcg ctc gag **cca tac tgg ctt cca aat ttc atg g** 3' (SEQ ID NO 5)

hCNA 5' PCR Oligo Start at Residue 350

Junk Xho1
5' atat aaa tcg ctc gag **ttt act tgg tcc ctt cca ttt gtt ggg g** 3' (SEQ ID NO 6)

hCNA 3' PCR Oligo End at Residue 370

Junk See Note ApaI Junk SaII
5' Cna gta ggg tct aga tct ggg ccc acg ata taa gtc gac **gtt gag gac**
att tac cag C 3' (SEQ ID NO 7)

Note: tct aga tct = overlapping XbaI and BglII sites. (SEQ ID NO 8)

hCNA 3' PCR Oligo End at Residue 394

See Note STP FLAG Peptide SaII
5' ttaa tct aga tct tca ctt gtc atc gtc atc ttt ata gtc gac **ctc**
ttt ccg ggc tgc agc tg 3' (SEQ ID NO 9)

Note: tct aga tct = overlapping XbaI and BglII sites. (SEQ ID NO 8)

• Beginning on page 80, line 9, (ending on page 81, line 3) replace the section in the specification with the following:

Oligos Designed for Human calcineurin B:
(**Bold** portion is CNB sequence)

hCNB 5' PCR Oligo Start at residue 2

Junk XhoI
5' atat aaa tcg ctc gag **gga aat gag gca agt tat cct ttg g** 3' (SEQ ID NO 10)

hCNB 5' PCR Oligo Start at residue 3

Junk XhoI
5' atat aaa tcg ctc gag **aat gag gca agt tat cct ttg g** 3' (SEQ ID NO 11)

hCNB 3' PCR Oligo with 3' FLAG peptide and Stop

See Note ApaI STP FLAG Peptide
5' ttaa tct aga tct ggg ccc tca ctt gtc atc gtc atc ttt ata
SaII
gtc gac **cac atc tac cac cat c** 3' (SEQ ID NO 12)

Note: tct aga tct = overlapping XbaI and BglII sites.

(SEQ ID NO 8)

- Beginning on page 81, line 6, (ending on page 82, line 13) replace the section in the specification with the following:

Oligos Designed for Constructing CNA-CNB Linkers:

(Bold portion is CNA sequence)

3' PCR Primer for hCNA to Generate 24 Amino Acid Template Linker (to residue 370)

Junk ApaI See Note Linker
5' cga ttt atat ggg ccc tct aga tct aga acc aga acc aga acc aga

Linker
acc aga acc aga acc aga acc aga acc aga acc acc aga acc aga acc

acc **gtt gag gac att tac cag c** 3'

(SEQ ID NO 13)

Note: tct aga tct = overlapping XbaI and BglII sites.

(SEQ ID NO 8)

3' PCR Primer for Randomizing the Length of the CNA-CNB Linker
(Register 1 oligo)

Junk See Note ApaI Junk SaI
5' g aat cgc aaa tct aga tct ggg ccc gtc atc ttt ata gtc gac acc

aga acc aga acc 3'

(SEQ ID NO 14)

Note: tct aga tct = overlapping XbaI and BglII sites.

(SEQ ID NO 8)

3' PCR Primer for Randomizing the Length of the CNA-CNB Linker
(Register 2 oligo)

Junk See Note ApaI Junk SaI
5' g aat cgc aaa tct aga tct ggg ccc gtc atc ttt ata gtc gac aga

acc aga acc aga 3'

(SEQ ID NO 15)

Note: tct aga tct = overlapping XbaI and BglII sites.

(SEQ ID NO 8)

CNA residue 370-

(SEQ ID NO 16)

(SEO ID NO 17)

(SEO ID NO 18)

(SEQ ID NO 19)

• Beginning on page 95, line 10, (ending on page 95, line 21) replace the paragraph in the specification with the following:

(SEO ID NO 22)

5

- Beginning on page 100, line 16, (ending on page 100, line 27) replace the paragraph in the specification with the following:

The following two oligonucleotides were phosphorylated with polynucleotide kinase, annealed, and ligated into pB42AD that had been digested with EcoRI and Xho I to give a new polylinker with the following restriction sites in order.

5' XhoI-Spacer-SalI-NcoI-BstEII-BspEI-AflIII-ApaI-EcoRI 3'

5' tcg acg aat tcg ggc ccc tta agt ccg gag gtc acc cat ggg tcg acg tcg gtc gta gac tcg aga 3'
(SEQ ID NO 23)

5' aat ttc tcg agt cta cga ccg acg tcg acc cat ggg tga cct ccg gac tta agg ggc ccg aat tcg 3'
(SEQ ID NO 24)

- Beginning on page 101, line 1, (ending on page 101, line 19) replace the section in the specification with the following:

The following oligos were used with standard PCR conditions to generate an FKBP fragment with a 5' EcoRI and a 3' BamHI. pLexA and this fragment were digested with EcoRI and BamHI, gel purified, and ligated.

FKBP oligos:

- FKBP Y2Hex 5' (5' oligo for FKBP with EcoRI and XhoI restriction sites

EcoRI XhoI Met
5' c ggg ccc ccc gaa ttc ctc gag atg ggc gtg cag gtg gag ac 3' (SEQ ID NO 25)

- FKBP Y2H^{sb} 3' (3' oligo for FKBP with 3'Sal I and BamHI restriction sites. No stop codon)

BamHI SalI E
5' ggg tct gga tcc gtg gac ttc cag ttt tag aag ctc g 3' (SEQ ID NO 26)

- Beginning on page 101, line 21, (ending on page 102, line 6) replace the section in the specification with the following:

Construction of the miniCABS for pLexA:

mCABS were PCR'd with standard PCR conditions off of my original miniCAB template with the following oligos and digested with NcoI and BamHI. This gel purified fragment was ligated to gel

purified pLexA digested with BamHI and NcoI.

3. mCAB Y2H^{ex} 5' (5' oligo for mCAB starting at residue 340 of CNA with 5' BamHI and XhoI sites _

BamHI XhoI Pro340

5' a tat aaa tgg gga tc cgt ctc gag cca tac tgg ctt cca aat ttc atg g 3' (SEQ ID NO 27)

4. mCAB Y2H^{stann} 3' (3' oligo for mCAB with 3' SalI, Flag, Stop, ApaI, NotI, and NcoI)

NcoI NotI ApaI stp -----Flag----- SalI

5' tct ttaa cca tgg cgg ccg c ggg ccc tca ctt gtc atc gtc atc ttt ata gtc gac cac atc tac
cac cat c 3' (SEQ ID NO 28)

• Beginning on page 102, line 27, (ending on page 103, line 17) replace the section in the specification with the following:

1. Overlap extension mutagenesis of the CNB portion of the mCABS.

5' oligo for generation of the N-terminal portion of CNB for overlap.

PflmI

5' ct tgg tcc ctt cca ttt gtt ggg gaa aaa gtg act gag 3' (SEQ ID NO 29)

3' oligo for generation of the N-terminal portion of CNB for overlap.

5' ggg aac aat ctg aaa gat aca cag tta cag c 3' (SEQ ID NO 30)

5' mutagenic oligo for generation of C-terminal portion of CNB for overlap.

Val119 Met118 Leu115

5' gctg taa ctg tgt atc ttt cag att gtt ccc (g/c)NN (g/c)NN cat ctt (g/c)NN tac ctg
gaa gag ttc ccc 3' (SEQ ID NO 31)

3' mutagenic oligo for generation of C-terminal portion of CNB for overlap.

XbaI BglIII ApaI Stp -----FLAG----- SalI 170

5' ttaa tct aga tct ggg ccc tca ctt gtc atc gtc atc ttt ata gtc gac cac atc tac cac
cat c 3' (SEQ ID NO 32)

• Beginning on page 103, line 19, (ending on page 103, line 30) replace the section in the specification with the following:

2. Mutagenic oligos for the CNA portion of mCABS.

5' Mutagenic oligo with 5' Xho I site.

XhoI 340
5' atat aaa tgg ctc gag cca tac tgg ctt cca aat ttc atg g 3' (SEQ ID NO 33)

3' Mutagenic oligo

PflMI 353 352
5' ctc agt cac ttt ttc ccc aac aaa tgg aag (g/c)NN (g/c)NN agt aaa aac atc cat g 3'
(SEQ ID NO 34)